

# Impacts of Drying and Rewetting on the Radiocarbon Signature of Respired $\text{CO}_2$ and Implications for Incubating Archived Soils

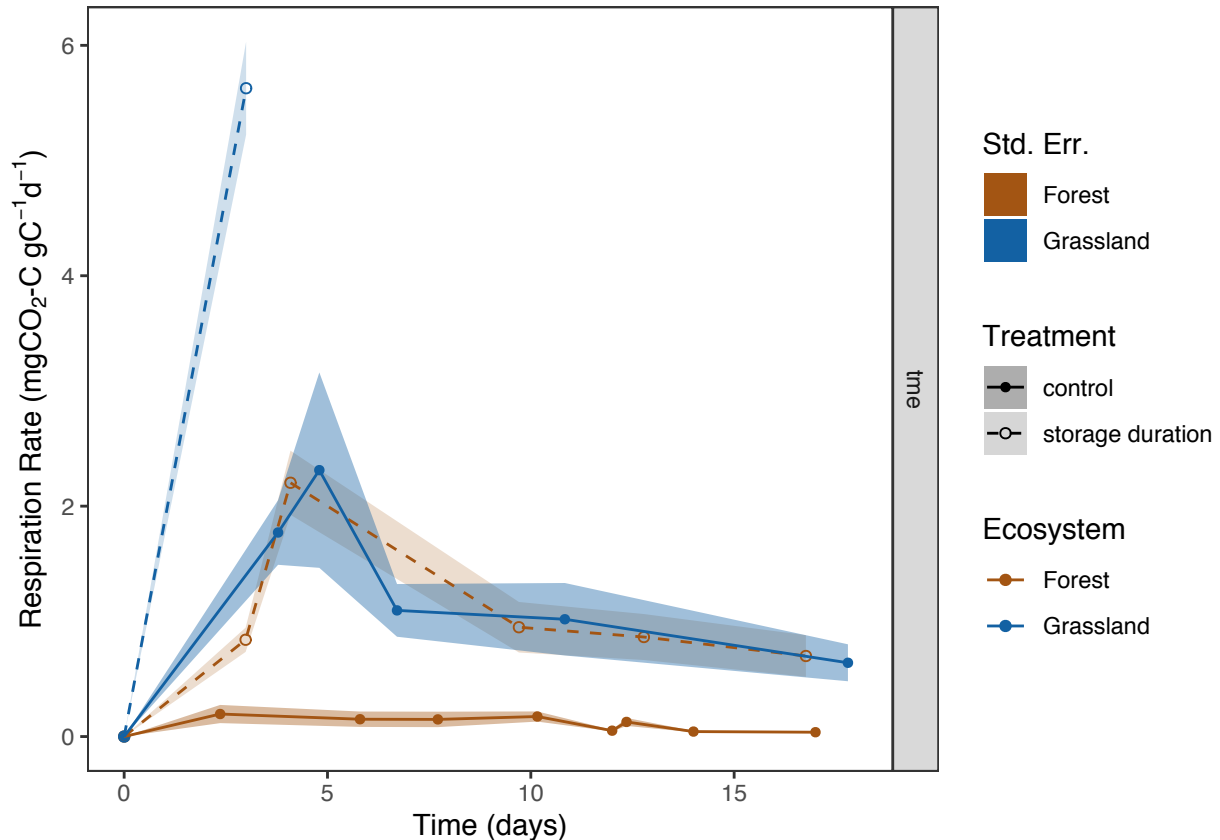
## Supplemental Figures

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### Supplemental respiration rates figures

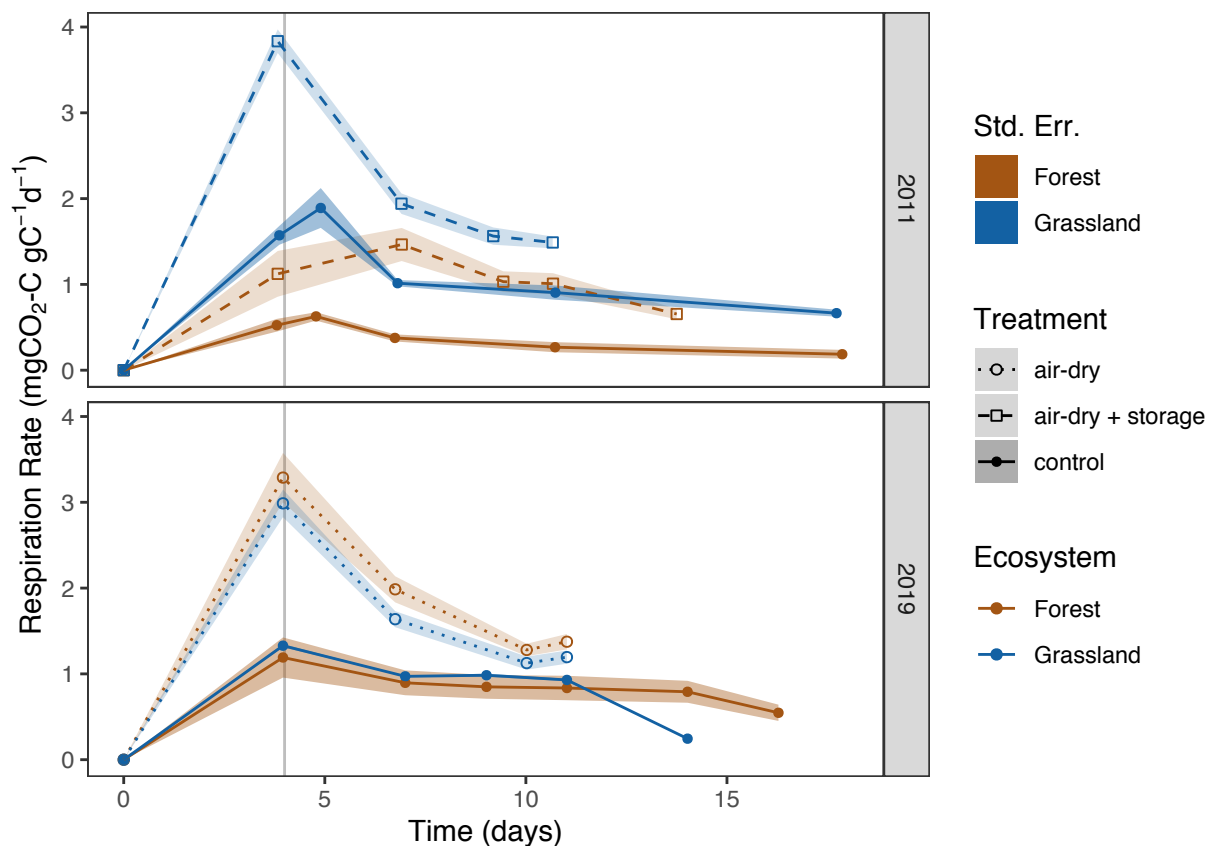
*Experiment 3 (storage duration)*



### Supplemental Fig 1. Respiration rates for Experiment 3

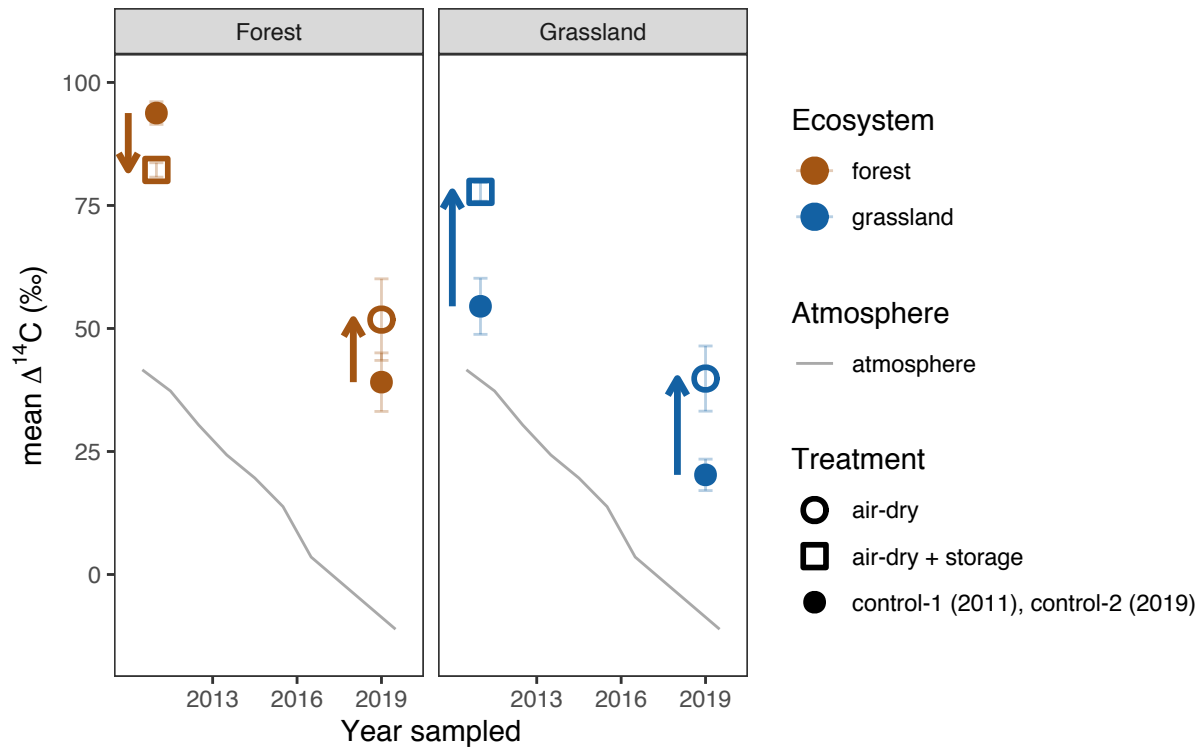
*Caption:* Experiment 3 storage duration treatment samples were only incubated for a single enclosure period, as the results of Experiment 1 and Experiment 2 showed no significant difference in  $\Delta^{14}\text{C}$ - $\text{CO}_2$  between the rewetting pulse  $\text{CO}_2$  released during the pre-incubation period and the  $\text{CO}_2$  respired during the equilibrium respiration period. The grassland storage duration treatment samples (blue dashed line) respired an equivalent amount of  $\text{CO}_2$  in just 3 d as the corresponding control-3 samples respired during the pre-incubation period and the equilibrium

respiration period combined. Consequently those incubations were stopped after the first CO<sub>2</sub> measurement point. Control-3 samples did undergo pre-incubation, but as the CO<sub>2</sub> release was not measured nor was  $\Delta^{14}\text{C-CO}_2$  for the majority of the samples, all data were averaged by day of measurement.

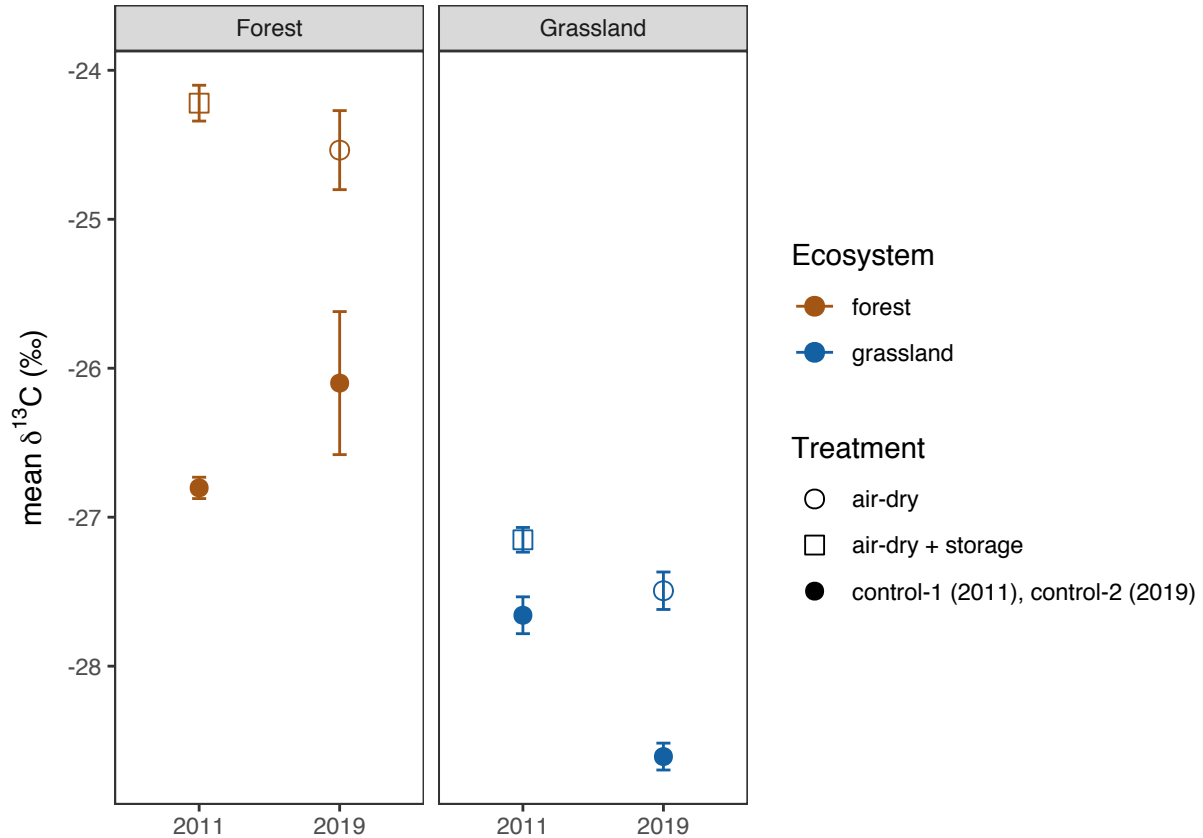


**Supplemental Fig 2. Respiration rates for Experiment 1 and Experiment 2 shown with all pre-incubation data calculated as cumulative averages**

*Caption:* CO<sub>2</sub> concentrations for Experiment 1 control samples were only measured once during the pre-incubation period, in contrast to daily measurements for all other samples. Pre-incubation respiration rates are shown here calculated as cumulative averages for the purpose of fair comparison across all treatments.



$\delta^{13}\text{C}$

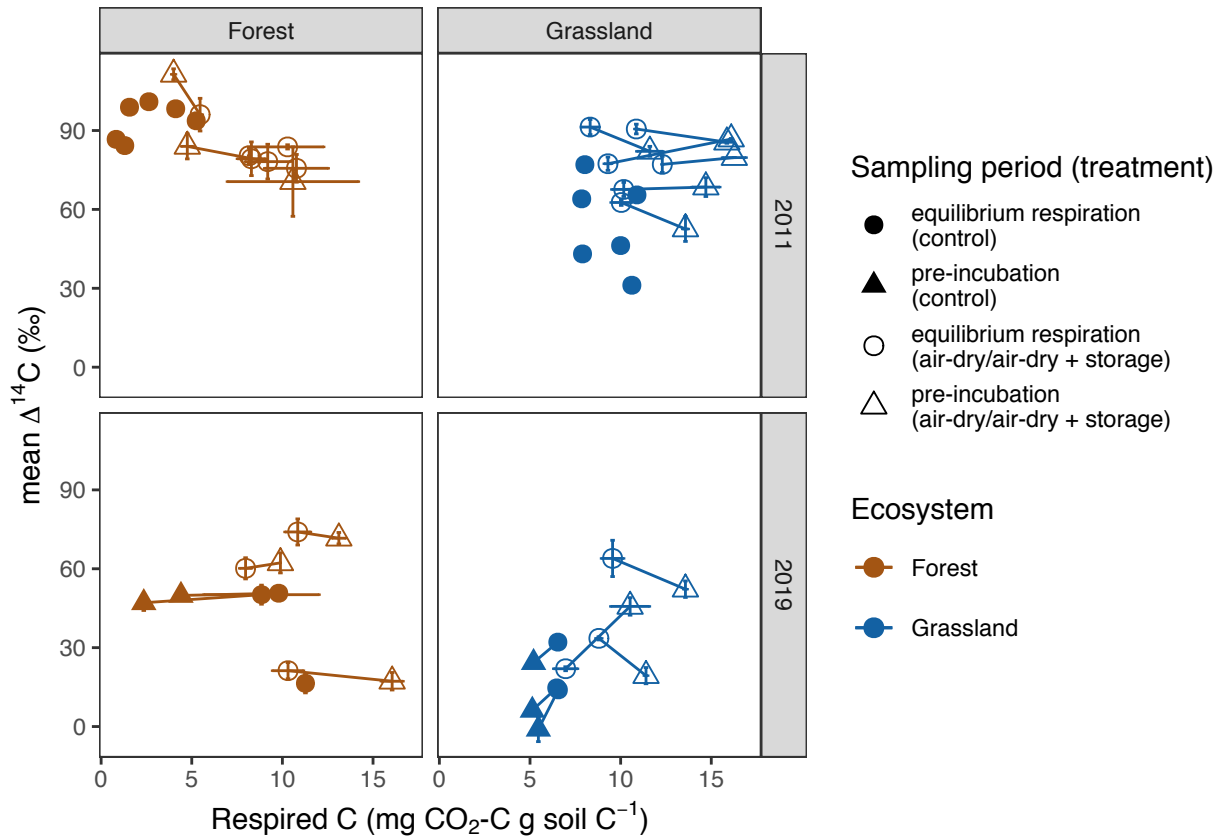


### Supplemental Fig 3. Time series of control and treatment $\delta^{13}\text{C}\text{-CO}_2$ (Experiments 1 and 2)

*Caption:* Filled circles show  $\delta^{13}\text{C}\text{-CO}_2$  observed for control samples, while open symbols show  $\delta^{13}\text{C}\text{-CO}_2$  observed for treatment samples (open squares = air-dry + storage treatment, Experiment 1, 2011; open circles = air-dry only treatment, Experiment 2, 2019). Points are means and error bars show 2x standard error.

### Treatment effect on $\Delta^{14}\text{C}$ as a function of the amount of carbon respired

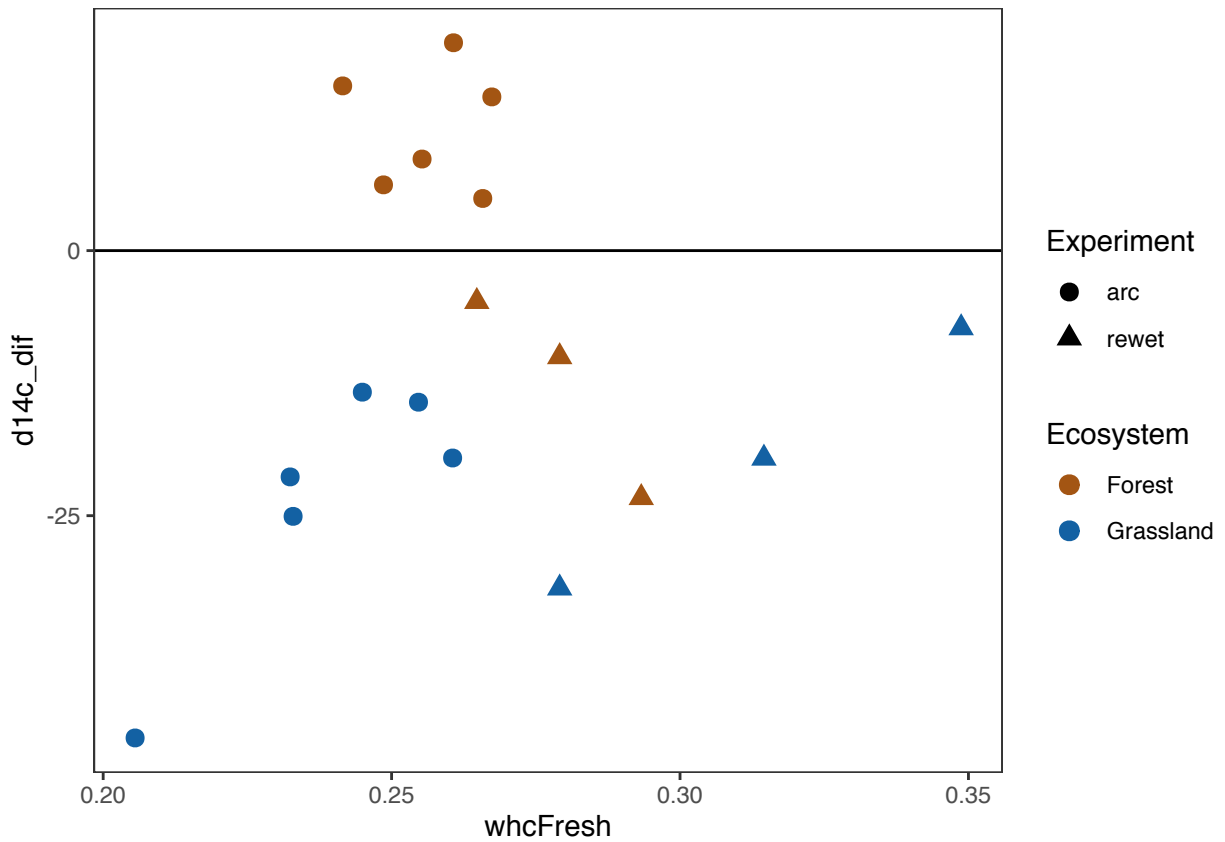
We looked at the possible effect of the difference in the amount of carbon respired ( $\text{mg CO}_2\text{-C g soil C}^{-1}$ ) on the differences between control and treatment  $^{14}\text{C}\text{-CO}_2$  using a linear regression model, but it was not significant overall. When data from Experiment 1 and Experiment 2 were considered separately, we observed a slight positive trend between the difference in respired carbon and the difference in  $^{14}\text{C}\text{-CO}_2$  within Experiment 2, but it was only marginally significant ( $p = 0.063$ ).



**Supplementary Fig. 4. Change in  $^{14}\text{C}\text{-CO}_2$  in relation to cumulative soil carbon respired**

*Caption:* Note that pre-incubation  $\Delta^{14}\text{C}$  was not measured for the control-1 samples in 2011. Limits exclude outlier point (HEW22 control-2, pre-incubation) for improved legibility. Points are means, error bars show min and max of duplicate samples.

# Treatment effect on $\Delta^{14}\text{C}$ as a function of field-moisture content



**Supplemental Fig 5. Change in  $\Delta^{14}\text{C}\text{-CO}_2$  (control - treatment) relative to field moisture**

*Caption:* Data are from Experiment 1 (“arc”) and Experiment 2 (“rewet”). All samples were moisture-adjusted prior to incubation, but control samples were adjusted from field moisture, “whcFresh” (percent of WHC), whereas treatment samples were moisture adjusted after air-drying, i.e. at approximately 0% of WHC.